DNA methylation-based classification of ependymomas in adulthood: implications for diagnosis and treatment

Witt, Hendrik ; Gramatzki, Dorothee ; Hentschel, Bettina ; et al ; Weller, Michael ; German Glioma Network

Abstract: Background Ependymal tumors are glial tumors that commonly manifest in children and young adults. Their classification has remained entirely morphological until recently, and surgery and radiotherapy are the main treatment options, especially in adults. Here we sought to correlate DNA methylation profiles with clinical and pathological characteristics in the prospective cohort of the German Glioma Network. Methods Tumors from 122 adult patients with myxopapillary ependymoma, ependymoma, anaplastic ependymoma, subependymoma or RELA fusion-positive ependymoma classified according to the World Heath Organization classification (WHO) 2016 were subjected to DNA methylation profiling using the Illumina HumanMethylation450 BeadChip platform. Molecular data were correlated with histologic features and clinical characteristics. Results At a median follow-up of 86.7 months, only 22 patients experienced progression (18.0%) and 13 patients (10.7%) died. Each tumor could be assigned to one of the previously defined molecular ependymoma subgroups. All histologic subependymomas corresponded to subependymoma (SE) DNA methylation subgroups, but the reverse was not true: 19 histologic ependymomas (WHO grade II) were allocated to molecular SE groups. Similarly, all histological myxopapillary ependymomas were assigned to the molecularly defined SP-MPE class, but this molecular subgroup additionally included 15 WHO grade II ependymomas by histology. Overall, WHO grade II ependymomas distributed into seven molecular subgroups. Conclusions Most adult patients with ependymoma show a favorable prognosis. Molecular classification may provide diagnostic and prognostic information beyond histology and facilitate patient stratification in future clinical trials. The prognostic significance of a subependymoma or myxopapillary ependymoma DNA methylation phenotype without according histology requires further study.

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DNA methylation-based classification of ependymomas in adulthood: implications for diagnosis and treatment

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- Ependymoma diagnosed in adult patients show most often a good prognosis.
- Molecular classification can support diagnostic and prognostic information beyond histology.

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Abstract:

Background:

Ependymal tumors are glial tumors that commonly manifest in children and young adults. Their classification has remained entirely morphological until recently, and surgery and radiotherapy are the main treatment options, especially in adults. Here we sought to correlate DNA methylation profiles with clinical and pathological characteristics in the prospective cohort of the German Glioma Network.

Methods:

Tumors from 122 adult patients with myxopapillary ependymoma, ependymoma, anaplastic ependymoma, subependymoma or RELA fusion-positive ependymoma classified according to the World Heath Organization classification (WHO) 2016 were subjected to DNA methylation profiling using the Illumina HumanMethylation450 BeadChip platform. Molecular data were correlated with histologic features and clinical characteristics.

Results:

At a median follow-up of 86.7 months, only 22 patients experienced progression (18.0%) and 13 patients (10.7%) died. Each tumor could be assigned to one of the previously defined molecular ependymoma subgroups. All histologic subependymomas corresponded to subependymoma (SE) DNA methylation subgroups, but the reverse was not true: 19 histologic ependymomas (WHO grade II) were allocated to molecular SE groups. Similarly, all histological myxopapillary ependymomas were assigned to the molecularly defined SP-MPE class, but this molecular subgroup additionally included 15 WHO grade II ependymomas by histology. Overall, WHO grade II ependymomas distributed into seven molecular subgroups.

Conclusions:

Most adult patients with ependymoma show a favorable prognosis. Molecular classification may provide diagnostic and prognostic information beyond histology and facilitate patient stratification in future clinical trials. The prognostic significance of a subependymoma or myxopapillary ependymoma DNA methylation phenotype without according histology requires further study.

Importance of the Study

This study used DNA methylation profiling to molecularly classify 122 adult ependymal tumors from patients enrolled in the German Glioma Network. Each tumor was assigned to one of 8 previously defined molecular subgroups of ependymal tumors. Histologically diagnosed subependymomas (SE), myxopapillary ependymomas (SP-MPE) and supratentorial RELA fusion-positive ependymomas (ST-EPN-RELA) were unambiguously assigned to the respective molecular subgroups. Only one anaplastic ependymoma (WHO grade III) was classified into the subgroup spinal subependymoma (SP-SE), the remaining six anaplastic ependymomas were assigned to the molecular subgroup posterior fossa group B (PF-EPN-B). WHO grade II ependymomas distributed into seven molecular subgroups. Future studies need to determine whether molecular reclassification allows for treatment de-escalation, e.g. delay of radiotherapy, in subgroups of patients. DNA methylation profiling may provide diagnostic and prognostic information beyond histology and thus may facilitate patient stratification in future clinical trials.

Introduction

The 2016 World Health Organization (WHO) classification of central nervous system tumors recognizes five distinct entities of ependymal tumors, subependymoma (WHO grade I), myxopapillary ependymoma (WHO grade I), ependymoma (WHO grade II), anaplastic ependymoma (WHO grade III), and RELA fusion-positive ependymoma (WHO grade II or III) [1]. Recently nine molecular subgroups of ependymomas with key genetic und epigenetic characteristics across all age groups have been described [2]. These were based on major central nervous system (CNS) compartments and histopathological and molecular findings: spine (SP), posterior fossa (PF) and supratentorial (ST) localization; subependymoma (SE), myxopapillary ependymoma (MPE), ependymoma (EPN), anaplastic ependymoma (EPN-A or -B: balanced genome or chromosomal instability), and ST-EPN-RELA, defined by *RELA* fusion transcript expression, as well as ST-EPN-YAP1, defined by the presence of *YAP1* fusion transcripts.

The current histopathologic assessment has shortcomings, reliability and clinical significance of WHO grade II versus III have remained controversial, and histologic subtyping provides only limited guidance for clinical decision making. It might thus be worthwhile to supplement the current WHO classification by assessment of additional molecular markers or large-scale molecular profiling approaches to tailor management strategies and to avoid under-treatment as well as over-treatment of individual patients. Accordingly, we molecularly classified ependymomas of adults based on DNA methylation patterns determined by 450k DNA methylation arrays and related these data to age, tumor location and histology. Longer follow-up of our cohort will be required to derive robust conclusions on the prognostic value of this new classifier in adult ependymoma patients.

Patients and methods

Molecular classification using DNA methylation profiling

The present study evaluated clinical features and histopathologic findings in the ependymoma cohort of the German Glioma Network (GGN). The GGN is a prospective cohort study that enrolled adult patients with gliomas at 9 clinical centers in Germany from 2004-2012 (http://www.gliomnetzwerk.de). All patients gave written informed consent according to the research proposals approved by the Institutional Review Boards of the participating institutions. We included all patients with newly diagnosed tumors histologically diagnosed as subependymoma, myxopapillary ependymoma, ependymoma or anaplastic ependymoma. The histologic diagnosis was verified by central neuropathology review in all 122 patients based on the 2016 WHO classification of tumors of the central nervous system [1].

The Illumina Infinium HumanMethylation450 (450k) array was used to obtain genome-wide DNA for tumor samples and normal control tissues (Illumina, San Diego, CA, USA). Data were generated at the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ, Heidelberg, Germany). DNA methylation data was generated from either fresh-frozen (n=88) or formalin-fixed paraffin-embedded (FFPE) tissue samples (n=34). Tumor tissue originated from primary tumors (n=114) and from relapse tumors (n=8). For most fresh-frozen samples, >500 ng of DNA was used as input material; 250 ng DNA was used for most FFPE tissues. On-chip quality metrics of all samples were carefully controlled. Samples were also checked for unexpected genotype matches by pairwise comparison of the 65 genotyping probes included on the 450k array.

All computational analyses were performed in R version 3.2.0 [3]. Raw signal intensities were obtained from IDAT-files using the minfi Bioconductor package

version1.14.0 [4]. Each sample was individually normalized by performing a background correction (shifting of the 5% percentile of negative control probe intensities to 0) and a dye-bias correction (scaling of the mean of normalization control probe intensities to 10,000) for both color channels. Subsequently, a correction for the type of material tissue (FFPE/frozen) was performed by fitting univariate, linear models to the log2-transformed intensity values (removeBatchEffect function, limma package version 3.24.15). The methylated and unmethylated signals were corrected individually. Beta-values were calculated from the retransformed intensities using an offset of 100 (as recommended by Illumina).

The following filtering criteria were applied: removal of probes targeting the X and Y chromosomes (n=11,551), removal of probes containing a single nucleotide polymorphism (dbSNP132 Common) within five base pairs of and including the targeted CpG site (n=7,998), probes not mapping uniquely to the human reference genome (hg19) allowing for one mismatch (n=3,965), and probes not included on the new Illumina EPIC array (n=32,260). In total, 428,799 probes were kept for the analysis.

Copy number variation (CNV) analysis from 450k methylation array data was performed using the conumee Bioconductor package version 1.3.0. Two sets of 50 control samples displaying a balanced copy number profile from both male and female donors were used. For CNV analysis no previous normalization steps were performed. To predict molecular subgroups a Random Forest (RF) classifier was applied using 428,799 probes. This classifier was trained on a reference set of 2,801 methylation profiles of brain tumors that were previously assigned to 91 molecular subgroups which cover almost all entities enlisted in the 2016 WHO classification of CNS tumors. For subgrouping of the 122 samples of this study the classification model including 91 molecular subgroups of brain tumors were used. The overall

prediction performance of this classifier was validated by a three-fold cross validation indicating a very high classification accuracy with a misclassification error rate of 4.28% and a multiclass AUC of 0.9998 [5,6].

Statistical analyses

Progression-free (PFS) and overall survival (OS) curves were estimated by the Kaplan-Meier method. PFS was calculated from the date of surgery to the date of progression. Patients without documented progression were censored at the last follow-up visit for PFS. OS was measured from the date of surgery to the date of death. Patients without confirmed death were censored for OS at last follow-up. Survival-related analyses were calculated using log-rank test. The association between molecular subgroups and clinical characteristics was analyzed by Chi-square test and Fisher's exact test and differences in age by Mann-Whitney U-test. All statistical tests were two-tailed, a p-value of 0.05 was set as statistically significant. All statistical analyses were performed using IBM SPSS Statistics Version 24. Chi-square tests were used to calculate the independence of distinct chromosomal aberrations across molecular subgroups [2]. Only gains and losses of whole chromosomes and chromosome arms were included into this analysis. Independences of CNV between molecular subgroups were calculated by Chi-square tests, p-values were computed by 100,000 Monte Carlo simulations.

Results

Patient characteristics

Table 1 summarizes relevant patient characteristics. Individual patient data are provided in Supplementary Table 1. Median age was 46 years (range: 18-80 years). The distribution of ages at diagnosis was relatively uniform across 10-year age groups between 30 and 70 years (15.6-18.9%), with a peak incidence in the 41-50 year age group (28.7%) and fewer diagnoses in patients older than 70 years (4.9%). There was a male predominance (67.2%). Histologic diagnosis was WHO grade II ependymoma in the majority of cases (60.7%). WHO grade III (anaplastic) ependymomas were rarely diagnosed. The most commonly affected compartment was the spine (53.3%) followed by infratentorial (31.1%) and supratentorial (14.8%) locations. Metastatic dissemination at diagnosis was observed only once. Patients who received therapy beyond surgery at first-line treatment had myxopapillary ependymoma (n=1), ependymoma WHO grade II (n=7), anaplastic ependymoma WHO grade III (n=5) or RELA fusion-positive ependymoma (n=2). Each of these patients was treated with postoperative radiotherapy (RT) (Table 2, Supplementary Table 1).

Median follow-up was 86.7 months, 22 patients experienced disease progression (18.0%), and 13 patients (10.7%) died, four patients with spinal, eight patients with intracranial and one patient with disseminated disease. Progression was seen in one of 23 patients (4.3%) with subependymoma, five of 14 patients (35.7%) with myxopapillary ependymoma, nine of 74 patients (12.2%) with ependymoma WHO grade II, four of seven patients (57.1%) with anaplastic ependymoma WHO grade III, and three of four patients with RELA fusion-positive tumors. At recurrence, 15 of 22 patients (68.2%) had a second resection, followed by RT (n=5), CCNU chemotherapy

(CT) (n=1), or combined radiochemotherapy with temozolomide (n=1). Two of 22 patients (9.1%) received RT alone and four patients received no further treatment (Table 2, Supplementary Table 1). PFS rate was 96.4% at 1 year (95% CI 93.0-100), 90.4% at 3 years (95% CI 84.6-96.1), and 84.6% at 5 years (95% CI 77.3-81.8) for the entire patient cohort. OS rate was 99.1% at 1 year (95% CI 97.5-100), 97.1% at 3 years (95% CI 93.9-100), and 95.9% at 5 years (95% CI 91.9-100). Deceased patients who did not die from ependymoma had other tumors (n=2) or cardiovascular or respiratory diseases (n=4).

Molecular subgrouping

Ependymal tumors of all 122 patients could be assigned to one of eight subgroups defined by distinct DNA methylation profiles: SP-SE (n=5; 4.1%), SP-MPE (n=29; 23.8%), SP-EPN (n=32; 26.2%), PF-SE (n=24; 19.7%), PF-EPN-A (n=1; 0.8%), PF-EPN-B (n=13; 10.7%), ST-SE (n=14; 11.5%) and ST-EPN-RELA (n=4; 3.3%) (Table 3, Supplementary Figure 1, 2). No tumor was assigned to another non-ependymal tumor entity and no tumor was removed from the analysis. Patients with PF-EPN-B and ST-EPN-RELA as well as with SP-MPE and SP-EPN tumors were younger (median age 37-43 years), whereas patients in the subgroups SP-SE, PF-SE, and ST-SE tended to be older (median age 49-59 years) (Supplementary Figure 3).

Figure 1 and Supplementary Table 2 illustrate how WHO 2016 diagnoses distribute into molecular subgroups and localization. All histologic subependymomas were assigned to the molecular SE groups. However, tumors assigned to the molecular subependymoma subgroups SP-SE, PF-SE and ST-SE also included 19 tumors histologically classified as ependymoma WHO grade II and one anaplastic ependymoma. All patients diagnosed with myxopapillary ependymoma were assigned to the molecularly defined SP-MPE subgroup. This molecular subgroup,

however, also included 15 WHO grade II ependymomas by histology. All patients assigned to SP-EPN, PF-EPN-A and PF-EPN-B corresponded to WHO grade II or III ependymomas (Figure 1, Supplementary Table 2).

The potential clinical implications become apparent when considering group assignments by compartment. This yielded few apparent changes in the supratentorial compartment (Figure 1a). Among the posterior fossa tumors, subependymomas and anaplastic ependymomas showed a perfect match into the PF-SE and PF-EPN-B groups whereas WHO grade II ependymomas were heterogeneous by DNA methylation profiling and distributed to the prognostically different groups of PF-SE and PF-EPN-B (Figure 1b). The most extensive reassignments by molecular profiling occurred in the spinal compartment where a third of WHO grade II ependymomas were assigned to the SP-MPE molecular subgroup (Figure 1c). Representative histologic patterns of tumors, which were assigned to distinct molecular subgroups such as subependymoma, ependymoma WHO grade II, myxopapillary ependymoma, anaplastic ependymoma, and *RELA* fusion-positive ependymoma, are shown in Supplementary Figure 4.

Chromosomal alterations show distinct patterns within molecular subgroups

CNVs were calculated by analyzing combined intensity values of the methylation probes [7]. Whole genome copy number profiles showed distinct chromosomal aberrations in terms of frequencies and specificity across the molecular ependymal subgroups (Figure 2, Supplementary Table 3). The single PF-EPN-PFA tumor was excluded here. Overall, molecular subependymoma (SE) group tumors within spinal, posterior fossa and supratentorial compartments showed infrequent CNVs. Surprisingly, SE commonly showed a loss of chromosome 19, most frequently within

PF-SE (79%), but also in ST-SE (50%) and SP-SE (40%) whereas genomic profiles were otherwise relatively flat. Another CNV frequently observed in SP-SE and PF-SE was partial chromosome 6 loss.

Chromosome 6 loss was also frequently seen in PF-EPN-B tumors (61%). Additionally, PF-EPN-B tumors showed gains of chromosomes 15 (54%), 18 (54%), and 20 (54%), and losses of chromosome 17 (38%) which were the most frequent aberrations [2,8,9]. Gain of chromosome 1q was seen in PF-EPN-B (23%) and in one ST-EPN-RELA.

In SP-EPN and SP-MPE, most chromosomal gains and losses comprised large regions including whole chromosomes or chromosomal arms. SP-EPN showed deletions of chromosome arm 22q in more than 80% of tumors [10]. Several CNVs were present in both SP-MPE and SP-EPN, the most common being loss of 22q in SP-EPN (90%) and SP-MPE (47%) tumors.

Characteristic and significant CNVs for SP-SE were losses of chromosomes 18 (20%) and 19 (40%), for SP-EPN gain of chromosome 12 (56%), loss of 13q (31%), and loss of 14q (31%). SP-MPE harbored gains of chromosome 16 (25%) and losses of chromosome 10 (25%). Exclusive chromosomal changes of ST-SE were losses of chromosomes 8 (29%) and 19 (50%). Characteristic and most frequent CNVs of ST-EPN-RELA tumors were losses of chromosome 3 (75%), 9 (100%) and 11 (75%), as well as focal losses of chromosome 11q (75%), where the fusion-partner genes *C11orf95* and *RELA* are localized [11,12].

Molecular subgroups and survival

Survival analyses showed differences in PFS and OS, although the low number of events allowed no conclusive statistical analysis between the eight molecular subgroups. Molecular subgroups associated with the poorest outcome were PF-EPN-

B and ST-EPN-RELA, with 5-year PFS probability of 65.8% for PF-EPN-B and 25% for ST-EPN-RELA patients (Supplementary Figures 5, 6).

The eight PF-EPN-B patients who had RT after initial surgery had a 5-years PFS rate of 72.9% (95% CI 40.6-100%) after a median follow-up of 7.3 years, as opposed to 53.3% (95% CI 4.7-100%) after a median follow-up of 8.5 years for patients who did not. Four of eight patients in the RT group relapsed compared to three out of five patients without further therapy. All patients in the latter group initially had a gross total resection. In the RT group only four of eight patients had been gross totally resected. The age was roughly equal (median 34.5 years for patients with RT versus 37 years for patients without treatment). PF-EPN-B patients had an OS rate at five years of around 100%, but survival probability dropped down to 37.5% at around 10 years (Supplementary Figure 5). No significant differences in PFS (p=0.468; data not shown) and OS (p=0.083; data not shown) between WHO grade II and III tumors were observed in this subgroup. Notably, three of six PF-EPN-B patients with WHO grade III tumors, but none of seven patients with WHO grade II died.

The number of ST-EPN-RELA tumors was small: three of four patients relapsed and died (Supplementary Figure 6). In contrast, SE tumors from the supratentorial and infratentorial compartments showed very good outcome. Importantly, two patients with ST-SE and one patient with PF-SE tumors died from other diseases, but not from their ependymomas (Supplementary Table 1).

Patients with spinal molecular subgroups SP-EPN, SP-MPE and SP-SE had an excellent OS rate of 100% after 5 years. SP-MPE may relapse earlier and more often than the other spinal molecular subtypes, but longer follow-up is needed (Supplementary Figure 7).

We investigated the molecular subgroup SP-MPE in more detail and compared patients with WHO grade I myxopapillary ependymoma (n=14) and WHO grade II

ependymoma (n=15), the latter representing patients whose tumors were molecularly reclassified as SP-MPE (Supplementary Table 4, Supplementary Figure 8). A comparison of clinical features revealed that patients with myxopapillary ependymomas WHO grade I were younger than patients with ependymomas WHO grade II (median 36 years versus 50 years, p=0.023). We observed a trend of SP-MPE-assigned WHO grade II ependymomas to show tumor localization of the conus medullaris or filum terminale (Supplementary Table 1). None of the patients with SP-MPE-assigned myxopapillary ependymomas died, but 2 patients with SP-MPE-assigned WHO grade II ependymomas did, one patient for unknown reason while death of the other patient was unrelated to the spinal tumor (Supplementary Table 4). For SP-MPE myxopapillary ependymoma patients 5 progressions were observed as compared to only one progression in patients with SP-MPE-assigned WHO grade II ependymoma (Supplementary Figure 8). The two cohorts of SP-MPE patients showed a trend for better PFS of patients with myxopapillary ependymomas (p=0.071, n.s).

The outcome comparison of the SP-EPN tumors (n=32), all of which had ependymoma WHO grade II histology, with tumors of the same histology that were assigned to the epigenetic SP-MPE subgroup (n=15) is presented in Supplementary Figure 9 and Supplementary Table 5. No relevant differences emerged. SP-EPN patients were younger by trend (median 42 years versus 50 years, p=0.123). Three patients died (n=1 SP-EPN and n=2 SP-MPE).

Discussion

Ependymomas are rare brain tumors in adults. Half of these tumors occur in the spinal cord. Surgery and radiotherapy are the main treatment modalities [13]. Gross total resection as safely feasible is recommended for all ependymal tumors whereas decisions against or for radiotherapy depend on residual tumor and histology based on the 2016 WHO classification [14].

The present study is the first effort to characterize adult ependymomas beyond the WHO classification 2016, using a classifier based on DNA methylation profiling recently proposed for a largely pediatric population [2,6]. The major strength of the present study is good clinical annotation regarding age, tumor location, treatment, and follow-up. Male patients were overrepresented in our cohort relative to data from larger registries [15]. The low number of PFS and OS events, despite long follow-up, reflects the improved contemporary outcome for adult ependymoma patients. However, it precluded an in depth analysis of the potential prognostic value of the new molecular classifier. Similarly, no firm conclusions on the role of therapeutic measures beyond surgery can be derived.

The vast majority of ependymomas in adults could~~an~~ readily be assigned to 7 of 9 recently defined molecular subgroups (Table 3). ST-EPN-YAP1 tumors were not detected. ST-EPN-RELA, ST-EPN-YAP1 and PF-EPN-A are more common in or even restricted to children. That none of the tumors included in this study were assigned a non-ependymoma diagnosis by methylation profiling may be explained by the central pathology review within the German Glioma Network that took place prior to inclusion.

The poor outcome of ST-EPN-RELA tumors observed in children may also be observed in adults (Table 3).

There was a weak trend in the small group of PF-EPN-B patients, who might show a better outcome when treated with radiotherapy compared to non-irradiated patients. However, due to the low number of patients, a recommendation of upfront radiation therapy cannot be concluded. If PF-EPN-B patients with incompletely resected tumors will benefit from upfront radiation therapy has to be evaluated in a randomized clinical trial in the future.

The most important potential clinical implications of our study can be deduced from Figure 1. First, although all histologically defined subependymomas of WHO grade I were assigned to the molecular subependymoma subgroups SP-SE, PF-SE and ST-SE, these molecular subgroups also included histologically defined ependymomas WHO grade II (n=19) and even a single case of anaplastic ependymoma WHO grade III. It is tempting to speculate, and supported by the preliminary data of this study, that among the histologically defined WHO grade II ependymomas, the molecular SE subgroup tumors have the best prognosis and patients with these tumors may be at risk of being overtreated with RT. The newly described chromosome 19 loss in subependymomas, especially in PF-SE, could potentially support the diagnostic process in the separation of PF-SE and PF-EPN-B, where this aberration was detected in less than 4% of cases (n=2) (Figure 2).

Second, while all patients with histologically defined myxopapillary ependymoma WHO grade I were assigned to the molecularly defined SP-MPE subgroup, this molecular subgroup additionally included 15 spinal WHO grade II ependymomas by histology. The prognostic significance of this change in group allocation from histology to molecular subgroup is less clear although some institutions adopt different post-resection strategies of wait-and-see versus radiotherapy in patients with myxopapillary ependymoma versus WHO grade II ependymoma [14]. Interestingly, gene expression profiling had previously indicated that WHO II grade ependymoma

and myxopapillary ependymoma are biologically distinct entities, although a subset of cases showed transcriptional profiles that could not be firmly assigned to either group [10]. The present study also suggests that a subset of spinal WHO grade II ependymomas is molecularly related to myxopapillary ependymomas by DNA methylation profiling. Interestingly, a previous study reported on the presence of both classic and myxopapillary histologic features in about 10% of lumbosacral ependymal tumors [16]. Thus, histology ~~and mRNA expression profiles~~ may be ambiguous or not fully representative in a subset of spinal ependymal tumors, and additional DNA methylation profiling may thus be helpful in refining the classification of these cases. In this context, DNA methylation patterns of brain tumors are usually stable from the cell of origin until the development of a brain tumor and even at relapse [2,17], ~~while gene expression changes may be more variable depending on microenvironmental influences as well as tumor cell differentiation and metabolism, as reported in spinal ependymomas [10].~~

Third, we hypothesize that once targeted treatments for ependymal tumors become available, a molecular classifier will be superior to histology to enrich for clinical trial populations of patients with tumors sharing a similar cell of origin and biology. ~~The verification of the *RELA fusion* can support the diagnostic process of supratentorial ependymoma.~~

Limitations of this study include the limited sample size per subgroup and the low number of PFS and OS events. Long-term follow up and independent cohort studies are required to confirm our assumption that molecular subgroup assignment of adult patients with ependymal tumors provides superior outcome description and enrichment for future clinical trials assessing therapeutic interventions.

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References

1. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016; 131(6):803-820.

2. Pajtler KW, Witt H, Sill M, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell.* 2015; 27(5):728-743.

3. R Development Core Team. *R: A language and environment for statistical computing.* . Vienna, Austria: R Foundation for Statistical Computing; 2015.

4. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics.* 2014; 30(10):1363-1369.

5. Breiman L. Random forests. *Machine Learning.* 2001; 45(1):5-32.

6. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature.* 2018; 555(7697):469-474.

7. Hovestadt V, Remke M, Kool M, et al. Robust molecular subgrouping and copy-number profiling of medulloblastoma from small amounts of archival tumour material using high-density DNA methylation arrays. *Acta Neuropathol.* 2013; 125(6):913-916.

8. Witt H, Mack SC, Ryzhova M, et al. Delineation of two clinically and molecularly distinct subgroups of posterior fossa ependymoma. *Cancer Cell.* 2011; 20(2):143-157.

9. Korshunov A, Witt H, Hielscher T, et al. Molecular staging of intracranial ependymoma in children and adults. *J Clin Oncol.* 2010; 28(19):3182-3190.

10. Mack SC, Agnihotri S, Bertrand KC, et al. Spinal Myxopapillary Ependymomas Demonstrate a Warburg Phenotype. *Clin Cancer Res.* 2015; 21(16):3750-3758.

11. Parker M, Mohankumar KM, Punchihewa C, et al. C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. *Nature.* 2014; 506(7489):451-455.

12. Pietsch T, Wohlers I, Goschzik T, et al. Supratentorial ependymomas of childhood carry C11orf95-RELA fusions leading to pathological activation of the NF-kappaB signaling pathway. *Acta Neuropathol.* 2014; 127(4):609-611.

13. Gilbert MR, Ruda R, Soffietti R. Ependymomas in adults. *Current neurology and neuroscience reports.* 2010; 10(3):240-247.

14. Ruda R, Reifenberger G, Frappaz D, et al. EANO guidelines for the diagnosis and treatment of ependymal tumors. *Neuro Oncol.* 2018; 20(4):445-456.

15. Vera-Bolanos E, Aldape K, Yuan Y, et al. Clinical course and progression-free survival of adult intracranial and spinal ependymoma patients. *Neuro Oncol.* 2015; 17(3):440-447.

16. Jeibmann A, Egensperger R, Kuchelmeister K, et al. Extent of surgical resection but not myxopapillary versus classical histopathological subtype affects prognosis in lumbo-sacral ependymomas. *Histopathology*. 2009; 54(2):260-262.

17. Hovestadt V, Jones DT, Picelli S, et al. Decoding the regulatory landscape of medulloblastoma using DNA methylation sequencing. *Nature.* 2014; 510(7506):537-541.

FIGURE LEGENDS

Figure 1. **Reassignment of WHO 2016-based ependymoma diagnoses to molecular ependymoma subgroups based on DNA methylation profiles in the GGN cohort of adult patients with ependymomas stratified according to tumor location.**

A, Supratentorial ependymomas (ST); B, Posterior fossa ependymomas (PF); C, Spinal ependymomas (SP).

Figure 2.

Copy number variations across molecular subgroups of adult ependymal tumors.

Summary of chromosomal imbalances showing distinct alterations within seven molecular subgroups of ependymal tumors. Copy number plots were generated based on DNA methylation, color code for losses (red), gains (green), and for balanced chromosomal profiles (gray). Results were plotted as frequencies at which these aberrations occurred within each molecular subgroup; significance is illustrated by p-values, which indicate distinct distributions of alterations across the molecular subgroups (chi-square test).

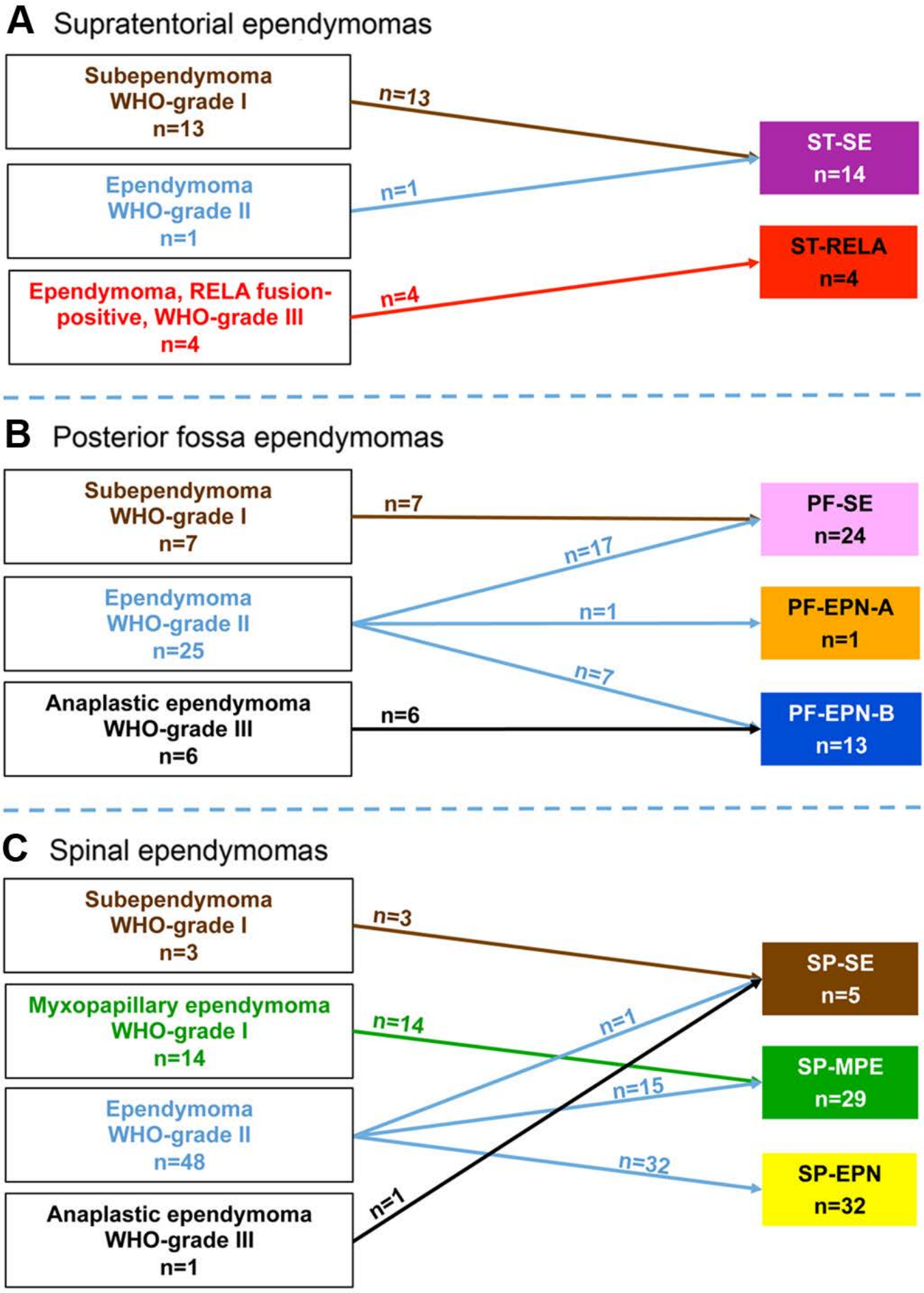
A Supratentorial ependymomas
Subependymoma WHO-grade I n=13
Ependymoma WHO-grade II n=1
Ependymoma, RELA fusion-positive, WHO-grade III n=4
n=13
n=1
n=4
ST-SE n=14
ST-RELA n=4
B Posterior fossa ependymomas
Subependymoma WHO-grade I n=7
Ependymoma WHO-grade II n=25
Anaplastic ependymoma WHO-grade III n=6
n=7
n=17
n=1
n=7
n=6
PF-SE n=24
PF-EPN-A n=1
PF-EPN-B n=13
C Spinal ependymomas
Subependymoma WHO-grade I n=3
Myxopapillary ependymoma WHO-grade I n=14
Ependymoma WHO-grade II n=48
Anaplastic ependymoma WHO-grade III n=1
n=3
n=14
n=1
n=15
n=32
n=1
SP-SE n=5
SP-MPE n=29
SP-EPN n=32

Figure 2:

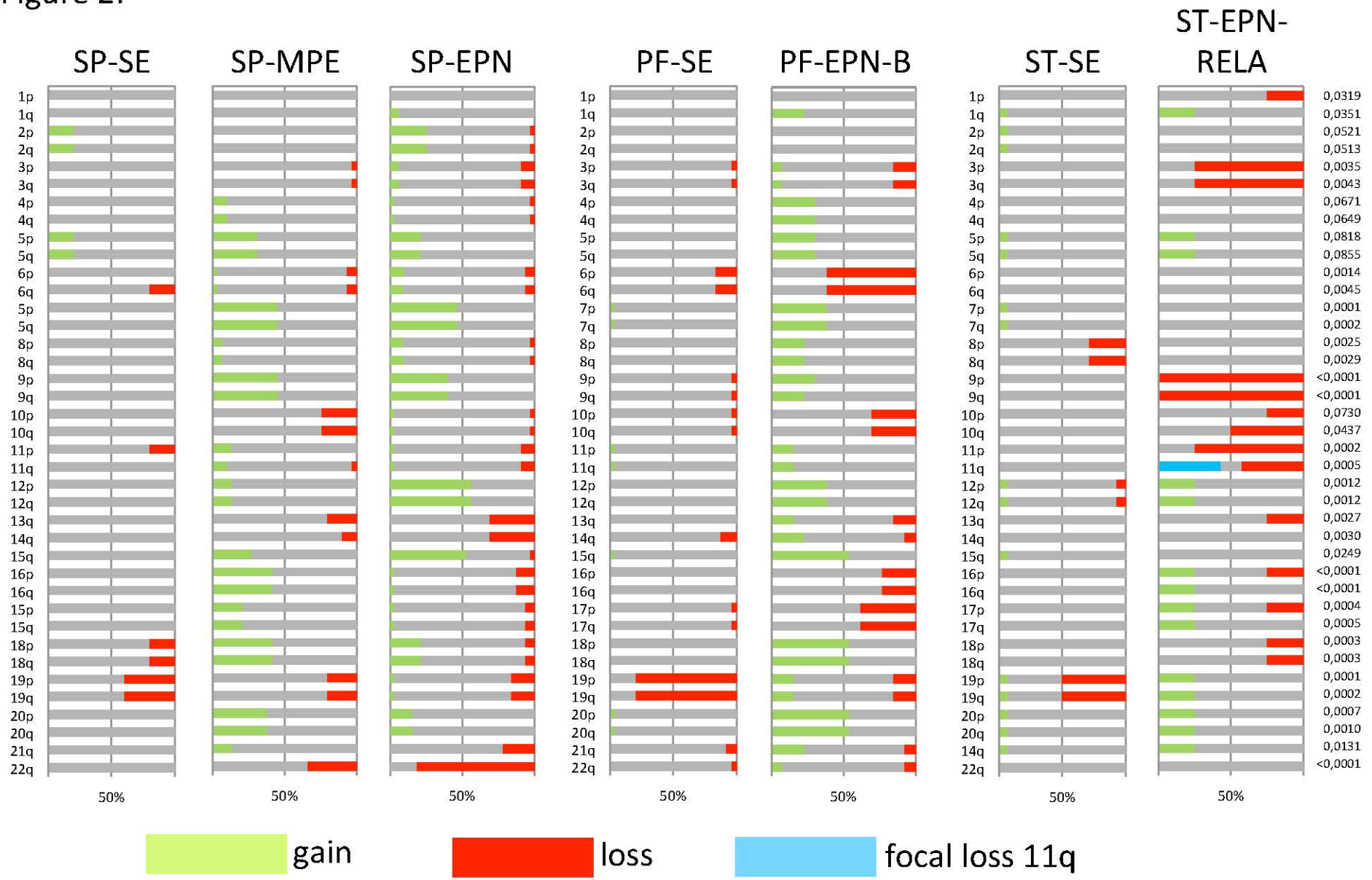
SP-SE
SP-MPE
SP-EPN
PF-SE
PF-EPN-B
ST-SE
ST-EPN-RELA
1p 1q 2p 2q 3p 3q 4p 4q 5p 5q 6p 6q 5p 5q 8p 8q 9p 9q 10p 10q 11p 11q 12p 12q 13q 14q 15q 16p 16q 15p 15q 18p 18q 19p 19q 20p 20q 21q 22q
1p 1q 2p 2q 3p 3q 4p 4q 5p 5q 6p 6q 7p 7q 8p 8q 9p 9q 10p 10q 11p 11q 12p 12q 13q 14q 15q 16p 16q 17p 17q 18p 18q 19p 19q 20p 20q 21q 22q
1p 1q 2p 2q 3p 3q 4p 4q 5p 5q 6p 6q 7p 7q 8p 8q 9p 9q 10p 10q 11p 11q 12p 12q 13q 14q 15q 16p 16q 17p 17q 18p 18q 19p 19q 20p 20q 14q 22q
0,0319 0,0351 0,0521 0,0513 0,0035 0,0043 0,0671 0,0649 0,0818 0,0855 0,0014 0,0045 0,0001 0,0002 0,0025 0,0029 <0,0001 <0,0001 0,0730 0,0437 0,0002 0,0005 0,0012 0,0012 0,0027 0,0030 0,0249 <0,0001 <0,0001 0,0004 0,0005 0,0003 0,0003 0,0001 0,0002 0,0007 0,0010 0,0131 <0,0001
50%
50%
50%
50%
50%
50%
50%
gain
loss
focal loss 11q

Table 1. Patient characteristics

	N (%)
All patients	**122**
Age (years)	
Median	46
Range	18 - 80
Age classes	
< 31 years	20 (16.4)
31 – 40 years	23 (18.9)
41 – 50 years	35 (28.7)
51 – 60 years	19 (15.6)
61 – 70 years	19 (15.6)
> 70 years	6 (4.9)
Gender	
Male	82 (67.2)
Female	40 (32.8)
KPS at enrolment	
<70	1 (1.0)
70-80	41 (40.6)
90-100	59 (58.4)
No data	21
Histological diagnosis (ICD-O)	
Subependymoma (9383/1)	23 (18.9)
Myxopapillary ependymoma (9394/1)	14 (11.5)
Ependymoma (9391/3; 9393/3)	74 (60.7)
Anaplastic ependymoma (9392/3)	7 (5.7)
RELA fusion-positive (9396/3)	4 (3.3)
WHO 2016 tumor grade	
I	37 (30.3)
II	74 (60.7)
III	11 (9.0)
Tumor localization at diagnosis	
Intracranial - supratentorial	18 (14.8)
Intracranial - infratentorial	38 (31.1)
Spinal	65 (53.3)
Disseminated	1 (0.8)
Follow-up	
Median follow-up (months)	86.7
Progression	22 (18.0)
Dead	13 (10.7)
Due to tumor progression	6 (4.9)
Other reason	6 (4.9)
Unknown	1 (0.8)

KPS: Karnofsky Performance Score; WHO: World Health Organisation.

Table 2. Treatment regimes

	N (%)
All patients	**122**
Initial tumor resection	
Partial	1 (0.8)
Subtotal	23 (18.9)
Gross total	98 (80.3)
Number of surgeries	
1	105 (86.1)
> 1	17 (13.9)
First-line therapy beyond surgery	
RT alone	15 (12.3)
No therapy	107 (87.7)
Therapy at first progression	22
No therapy	4 (18.0)
Re-resection	8 (36.4)
Re-resection plus CCNU	1 (4.5)
Re-resection plus RT	5 (22.7)
Re-resection plus TMZ/RT	1 (4.5)
RT	2 (13.6)

RT: radiotherapy; TMZ: temozolomide.

Table 3. Patient characteristics based on ependymal tumor subgroups

	SP-SE	SP-MPE	SP-EPN	PF-SE	PF-EPN-A	PF-EPN-B	ST-SE	ST-EPN-RELA
Number n (%)	5 (4.1)	29 (23.8)	32 (26.2)	24 (19.7)	1 (0.8)	13 (10.7)	14 (11.5)	4 (3.3)
Age (years; median range)	59 (46-73)	43 (18-80)	42 (24-69)	54 (18-79)	42 (42-42)	37 (19-63)	49 (26-73)	37 (26-62)
Gender								
Male	2 (40.0)	20 (69.0)	17 (53.1)	23 (95.8)	1 (100.0)	6 (46.2)	11 (78.6)	2 (50.0)
Female	3 (60.0)	9 (31.0)	15 (46.9)	1 (4.2)	0	7 (53.8)	3 (21.4)	2 (50.0)
KPS								
<70	0	0	0	1 (4.2)	0	0	0	0
70-80	2 (66.7)	11 (52.4)	10 (41.7)	8 (33.3)	1 (100.0)	3 (25.0)	6 (46.2)	0
90-100	1 (33.3)	10 (47.6)	14 (58.3)	15 (62.5)	0	9 (75.0)	7 (53.8)	3 (100.0)
No data	2	8	8			1	1	1
Surgery								
Total	4 (80.0)	25 (86.2)	28 (87.5)	17 (70.8)	0	9 (69.2)	12 (85.7)	3 (75.0)
Subtotal	1 (20.0)	4 (13.8)	3 (9.4)	7 (29.2)	1 (100.0)	4 (30.8)	2 (14.3)	1 (25.0)
Partial	0	0	1 (3.1)	0	0	0	0	0
Histology								
Subependymoma	3 (60.0)	0	0	7 (29.2)	0	0	13 (92.9)	0
Myxopapillary ependymoma	0	14 (48.3)	0	0	0	0	0	0
Ependymoma	1 (20.0)	15 (51.7)	32 (100.0)	17 (70.8)	1 (100.0)	7 (53.8)	1 (7.1)	0
Anaplastic ependymoma	1 (20.0)	0	0	0	0	6 (46.2)	0	4 (100.0)
RELA fusion-positive	0	0	0	0	0	0	0	4 (100.0)
WHO 2016 grade								
I	3 (60.0)	14 (48.3)	0	7 (29.2)	0	0	13 (92.9)	0
II	1 (20.0)	15 (51.7)	32 (100.0)	17 (70.8)	1 (100.0)	7 (53.8)	1 (7.1)	0
III	1 (20.0)	0	0	0	0	6 (46.2)	0	4 (100.0)
Localization								
Intracranial – supratentorial	0	0	0	0	0	0	14 (100.0)	4 (100.0)
Intracranial – infratentorial	1 (20.0)	0	0	24 (100.0)	1 (100.0)	12 (92.3)	0	0
Spinal	4 (80.0)	29 (100.0)	32 (100.0)	0	0	0	0	0
Disseminated	0	0	0	0	0	1 (7.7)	0	0
First-line therapy beyond surgery								
RT alone (including cyber knife)	0	2 (6.9)	1 (3.1)	2 (8.3)	0	8 (61.5)	0	2 (50.0)
None	5 (100.0)	27 (93.1)	31 (96.9)	22 (91.7)	1 (100.0%)	5 (38.5)	14 (100.0)	2 (50.0)
Progression								
Events, n (%)	0	6 (20.7)	2 (6.3)	3 (12.5)	0	7 (53.8)	1 (7.1)	3 (75.0)
Death								
Events, n (%)	0	2 (6.9)	1 (3.1)	2 (8.3)	0	3 (23.1)	2 (14.3)	3 (75.0)

KPS: Karnofsky Performance Score; WHO: World Health Organisation

Supplementary Tables

DNA methylation-based classification of ependymomas in adulthood: implications for diagnosis and treatment

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Supplementary Table 1.

Individual patient characteristics.

Patient	Age	Gender	Histology	WHO grade	Molecular subgroup	Tumor localisation	Extent of resection	Adjuvant therapy	PFS (months)	Salvage therapy	OS (months)
1	42	m	ependymoma	II	PF-EPN-A	intracranial - infratentorial	subtotal	none	108.2		108.2
2§§	30	f	anaplastic ependymoma *	III	PF-EPN-B	disseminated, brain stem	total	none	8.5+	re-resection	100.2++§
3	19	f	ependymoma	II	PF-EPN-B	intracranial - infratentorial	total	none	60.8+	re-resection plus RT	120.8
4	24	m	ependymoma	II	PF-EPN-B	intracranial - infratentorial	total	RT	62.1		62.1
5	28	f	ependymoma	II	PF-EPN-B	intracranial - infratentorial	subtotal	RT	15.4+	re-resection	167.1
6	37	m	ependymoma	II	PF-EPN-B	intracranial - infratentorial	total	none	16.8		16.8
7	41	f	ependymoma	II	PF-EPN-B	intracranial - infratentorial	total	RT	83.1		83.1
8	44	m	ependymoma	II	PF-EPN-B	intracranial - infratentorial	total	none	52.2+	re-resection plus RT	101.9
9	51	f	ependymoma	II	PF-EPN-B	intracranial - infratentorial	total	none	72.0		72.0
10	27	f	anaplastic ependymoma	III	PF-EPN-B	intracranial - infratentorial	total	RT	19.9		19.9
11	54	m	anaplastic ependymoma	III	PF-EPN-B	intracranial - infratentorial	total	RT	66.9		66.9++§
12§§	55	m	anaplastic ependymoma	III	PF-EPN-B	intracranial - infratentorial	subtotal	RT	30.4+	re-resection	87.5
13	63	f	anaplastic ependymoma	III	PF-EPN-B	intracranial - infratentorial	subtotal	RT	67.9+	re-resection plus RT	117.8

14	25	m	anaplastic ependymoma	III	PF-EPN-B	intracranial - infratentorial	subtotal	RT	66.7+	re-resection plus CCNU	128.4++
15	46	m	subependymoma	I	PF-SE	intracranial - infratentorial	total	none	81.5		81.5
16	48	m	subependymoma	I	PF-SE	intracranial - infratentorial	total	none	20.5		20.5
17	52	m	subependymoma	I	PF-SE	intracranial - infratentorial	subtotal	none	13.1		13.1
18	54	m	subependymoma	I	PF-SE	intracranial - infratentorial	total	none	116.5		116.5
19	56	m	subependymoma	I	PF-SE	intracranial - infratentorial	total	none	111.3		111.3
20	67	m	subependymoma	I	PF-SE	intracranial - infratentorial	total	none	33.7+	-	33.7++
21	35	m	subependymoma	I	PF-SE	intracranial - infratentorial	total	none	15.9		15.9
22	18	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	0.1		0.1
23	38	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	84.3		84.3
24	40	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	57.9		57.9
25	40	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	56.5		56.5
26	45	m	ependymoma	II	PF-SE	intracranial - infratentorial	subtotal	none	24.3		24.3
27	47	f	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	82.2		82.2
28	52	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	38.9		38.9
29	54	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	38.7		38.7
30	60	m	ependymoma	II	PF-SE	intracranial - infratentorial	subtotal	RT	89.3		89.3

31	62	m	ependymoma	II	PF-SE	intracranial - infratentorial	subtotal	none	71.9		71.9
32	65	m	ependymoma	II	PF-SE	intracranial - infratentorial	subtotal	none	46.6+	RT	49.8
33	65	m	ependymoma	II	PF-SE	intracranial - infratentorial	subtotal	none	114.8		114.8
34	66	m	ependymoma	II	PF-SE	intracranial - infratentorial	subtotal	RT	41.6+	RT	121.4
35	67	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	86.7		86.7
36	70	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	0.6		0.6++§
37	71	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	82.6		82.6
38	79	m	ependymoma	II	PF-SE	intracranial - infratentorial	total	none	79.6		79.6
39	24	f	ependymoma	II	SP-EPN	spinal	subtotal	none	36.5		36.5
40	29	f	ependymoma	II	SP-EPN	spinal	total	none	35.1		35.1
41	30	f	ependymoma	II	SP-EPN	spinal	total	none	141.2		141.2
42	31	m	ependymoma	II	SP-EPN	spinal	total	RT	53.4+	-	90.5
43	32	f	ependymoma	II	SP-EPN	spinal	total	none	85.3		85.3
44	32	m	ependymoma	II	SP-EPN	spinal	total	none	47.4		47.4
45	32	m	ependymoma	II	SP-EPN	spinal	total	none	105.9		105.9
46	34	m	ependymoma	II	SP-EPN	spinal	subtotal	none	30.2		30.2
47	36	f	ependymoma	II	SP-EPN	spinal	total	none	36.8		36.8
48	36	f	ependymoma	II	SP-EPN	spinal	total	none	14.9		14.9
49	38	m	ependymoma	II	SP-EPN	spinal	total	none	99.7		99.7
50	39	m	ependymoma	II	SP-EPN	spinal	total	none	33.3		33.3
51	39	m	ependymoma	II	SP-EPN	spinal	total	none	122.1		122.1
52	40	m	ependymoma	II	SP-EPN	spinal	total	none	0.1		0.1
53	41	m	ependymoma	II	SP-EPN	spinal	total	none	71.8		71.8

54	42	f	ependymoma	II	SP-EPN	spinal	total	none	58.1		58.1
55	42	f	ependymoma	II	SP-EPN	spinal	total	none	65.6		65.6
56	43	f	ependymoma	II	SP-EPN	spinal	total	none	180.5		180.5
57	44	f	ependymoma	II	SP-EPN	spinal	total	none	109.5		109.5
58	45	m	ependymoma	II	SP-EPN	spinal	total	none	95.8		95.8
59	46	f	ependymoma	II	SP-EPN	spinal	total	none	15.8		15.8
60	46	m	ependymoma	II	SP-EPN	spinal	subtotal	none	59.5		59.5
61	49	m	ependymoma	II	SP-EPN	spinal	total	none	98.5+	-	98.5++
62	49	f	ependymoma	II	SP-EPN	spinal	total	none	117.1		117.1
63	49	m	ependymoma	II	SP-EPN	spinal	total	none	131.7		131.7
64	50	m	ependymoma	II	SP-EPN	spinal	total	none	0.2		0.2
65	53	f	ependymoma	II	SP-EPN	spinal	total	none	32.3		32.3
66	60	f	ependymoma	II	SP-EPN	spinal	total	none	5.3		5.3
67	67	m	ependymoma	II	SP-EPN	spinal	total	none	136.4		136.4
68	69	f	ependymoma	II	SP-EPN	spinal	partial	none	43.5		43.5
69	69	m	ependymoma	II	SP-EPN	spinal	total	none	16.4		16.4
70	69	m	ependymoma	II	SP-EPN	spinal	total	none	73.3		73.3
71	23	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	47.0+	RT	65.0
72§§	26	m	myxopapillary ependymoma *	I	SP-MPE	spinal	subtotal	none	34.8+	re-resection	199.7
73	27	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	142.6		142.6
74	28	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	105.6		105.6
75	29	f	myxopapillary ependymoma	I	SP-MPE	spinal	total	RT	95.6		95.6
76	34	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	68.4		68.4
77§§	38	m	myxopapillary ependymoma *	I	SP-MPE	spinal	subtotal	none	13.4+	re-resection	124.2

78	43	f	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	125.4		125.4
79	44	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	0.3		0.3
80	49	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	149.7		149.7
81	50	m	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	75.3+	re-resection plus RT	114.5
82	67	f	myxopapillary ependymoma	I	SP-MPE	spinal	total	none	83.3		83.3
83	29	m	ependymoma	II	SP-MPE	spinal**	total	none	62.8		62.8
84	31	f	ependymoma	II	SP-MPE	spinal**	total	none	110.8		110.8
85	39	f	ependymoma	II	SP-MPE	spinal**	total	none	112.9		112.9
86	39	m	ependymoma	II	SP-MPE	spinal**	total	none	82.3		82.3
87	41	m	ependymoma	II	SP-MPE	spinal**	total	none	109.4		109.4
88	41	m	ependymoma	II	SP-MPE	spinal**	subtotal	none	22.8		22.8
89	50	m	ependymoma	II	SP-MPE	spinal**	total	none	129.5		129.5
90	50	f	ependymoma	II	SP-MPE	spinal**	total	none	100.1		100.1
91	52	m	ependymoma	II	SP-MPE	spinal**	total	none	47.6		47.6
92	54	m	ependymoma	II	SP-MPE	spinal**	total	none	124.7		124.7
93	57	f	ependymoma	II	SP-MPE	spinal**	total	none	54.0		54.0
94	58	m	myxopapillary ependymoma	I	SP-MPE	spinal**	total	none	105.4		105.4
95§§	59	f	ependymoma *	II	SP-MPE	spinal**	total	none	36.0+	re-resection	111.1
96	62	f	ependymoma	II	SP-MPE	spinal**	total	none	100.1		100.1
97	71	m	ependymoma	II	SP-MPE	spinal	total	RT	78.4		78.4++
98	80	m	ependymoma	II	SP-MPE	spinal**	total	none	79.9		79.9++§
99§§	18	m	myxopapillary ependymoma *	I	SP-MPE	spinal	subtotal	none	91.6+	re-resection	222.0
100	73	m	subependymoma	I	SP-SE	intracranial - infratentorial	total	none	116.4		116.4

101	46	f	subependymoma	I	SP-SE	spinal	total	none	115.3		115.3
102	65	m	subependymoma	I	SP-SE	spinal	subtotal	none	4.3		4.3
103	48	f	ependymoma	II	SP-SE	spinal	total	none	47.3		47.3
104	59	f	anaplastic ependymoma	III	SP-SE	spinal	total	none	31.1		31.1
105§§	28	f	RELA fusion-positive *	III	ST-EPN-RELA	intracranial - supratentorial	total	none	11.5+	re-resection plus RT	185.9++
106	26	m	RELA fusion-positive	III	ST-EPN-RELA	intracranial - supratentorial	total	none	6.8+	re-resection	122.9++
107	46	m	RELA fusion-positive	III	ST-EPN-RELA	intracranial - supratentorial	subtotal	RT	4.4+	re-resection plus RT/TMZ	18.2++
108	62	f	RELA fusion-positive	III	ST-EPN-RELA	intracranial - supratentorial	total	RT	107.2		107.2
109	26	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	4.7		4.7
110	34	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	18.1		18.1
111	41	f	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	104.1		104.1
112	45	f	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	24.3		24.3
113	46	m	subependymoma	I	ST-SE	intracranial - supratentorial	subtotal	none	136.8		136.8
114	48	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	116.9		116.9
115	50	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	109.3		109.3
116	54	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	0.2		0.2
117	56	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	4.0		4.0
118	64	m	subependymoma	I	ST-SE	intracranial - supratentorial	subtotal	none	121.7		121.7
119	64	f	subependymoma	I	ST-SE	intracranial -	total	none	104.3		104.3

						supratentorial					
120	73	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	59.0		59.0++§
121	32	m	subependymoma	I	ST-SE	intracranial - supratentorial	total	none	0.0		0.0
122§§	63	m	ependymoma *	II	ST-SE	intracranial - supratentorial	total	none	97.7+	-	170.7++§

m, male; f, female; RT, radiotherapy; PFS, progression-free survival; OS, overall survival; * histological diagnosis was verified by central neuropathological review from recurrent tumor tissue (n=7); + indicates patients with progressive disease; ++ indicates patients who are deceased ; § patient deceased but not because of ependymal tumor progression; ** located in the conus medullaris, cauda equina, or filum terminale (data were only assessed for patients diagnosed histopathologically with “ependymoma” and molecularly with “SP-MPE”). §§ indicates cases where recurrent tumor tissue was used for DNA methylation profiles.

Supplementary Table 2.
Histological association with molecular subgroup and tumor localization.

Histological diagnosis	Number (Localization)	Molecular Subgroup
Subependymoma **WHO-grade I** n=23	n=2 (spinal) n=1 (infratentorial) n=7 (infratentotrial) n=13 (supratentorial)	SP-SE SP-SE PF-SE ST-SE
Myxopapillary ependymoma **WHO-grade I** n=14	n=14 (spinal)	SP-MPE
Ependymoma **WHO-grade II** n=74	n=1 (supratentorial) n=1 (spinal) n=15 (spinal) n=32 (spinal) n=17 (infratentorial) n=7 (infratentorial) n=1 (infratentorial)	ST-SE SP-SE SP-MPE SP-EPN PF-SE PF-EPN-B PF-EPN-A
Anaplastic ependymoma **WHO-grade III** n=7	n=1 (brain stem, disseminated) n=5 (infratentorial) n=1 (spinal)	PF-EPN-B PF-EPN-B SP-SE
RELA-fusion positive **WHO-grade III** n=4	n=4 (supratentorial)	ST-EPN-RELA

Supplementary Table 3.
Frequency of copy number variations.

Molecular Subgroups	Chromosome gains	Chromosome losses
SP-SE	-	6 (20%), 18 (20%), 19 (40%)
SP-MPE	16 (25%)	10 (25%), 22q del. (47%)
SP-EPN	12 (56%)	13q (31%), 14q (31%), 22q del. (90%)
PF-SE	-	6 (17%), 19 (79%)
PF-EPN-B	1q (23%), 15q (54%), 18 (54%), 20 (54%)	6 (61%), 17 (38%)
ST-SE	-	8 (29%), 19 (50%)
ST-EPN-RELA	1q (25%)	3 (75%), 9 (100%), 11 (75%), focal loss 11q (75%)

del., deletion; only selected characteristic copy number variations were included in this table.

Supplementary Table 4. (related to Supplementary Figure 8)

Clinical features of molecularly classified spinal myxopapillary ependymoma (SP-MPE) by histology: histologicalmyxopapillary ependymoma (WHO grade I) versus histological spinal ependymoma (WHO grade II).

Molecular Subgroup Spinal Myxopapillary Ependymoma (SP-MPE) N=29 **----------** **Clinical information**	**Histological diagnosis Myxopapillary Ependymoma (WHO grade I) n=14**	**Histological diagnosis Ependymoma (WHO grade II) N=15**	**P-value**
Age, median (range)	36 year (18-67years)	50 years (29-80 years)	***0.023***
Male, n (%)	11/14 (78.6%)	9/15 (60.0%)	*0.427*
Gross total resection, n (%)	11/14 (78.6%)	14/15 (93.3%)	*0.330*
KPS <90, n (%)	4/8 (50%)	7/13 (53.8%)	*1.0*
First-line RT	 1/14 (7.1%)	 1/15 (6.7%)	 *1.0*
Progress	5/14 (35.7%)	1/15 (6.7%)	*0.08*
Salvage Re-resection Re-resection + RT RT	 3 1 1	 1 0 0	 *n.s.* *n.s.* *n.s*
Death	0/14 (0%)	2*/15 (13.3%)	*0.483*

P-values calculated by Fisher's exact test. *Cause of death of one patient was not brain tumor-related, for the other one unknown.

Supplementary Table 5 (related to Supplementary Figure 9).

Clinical features of histological spinal WHO grade II tumors, either molecularly classified as myxopapillary ependymoma (SP-MPE; n=15) or as spinal ependymoma (SP-EPN; n=32).

Histological diagnosis **Spinal WHO grade II ependymomas** **N=47** **----------** **Clinical information**	**Molecular Subgroup** **Spinal Myxopapillary Ependymoma (SP-MPE)** **only WHO grade II tumors** **n=15**	**Molecular Subgroup** **Spinal Ependymoma (SP-EPN)** **only WHO grade II tumors** **n=32**	**P-value**
Age, median (range)	50 years (29-80)	42 years (24-69)	0.123
Male, n (%)	9/15 (60.0%)	17/32 (53.1%)	0.659
Gross total resection	14/15 (93.3%)	28/32 (87.5%)	1.0
KPS <90%	7/13 (53.8%)	10/24 (41.7%)	0.478
First-line RT	 1/15 (6.7%)	 1/32 (3.1%)	 0.541
Progress	1/15 (6.7%)	2/32 (6.3%)	1.0
Salvage Re-resection None	 1 0	 0 0	
Death	2*/15 (13.3%)	1/32 (3.1%)	0.235

P-values calculated by Fisher's exact test. *Cause of death of one patient was not brain tumor related, for the other one unknown.

Supplementary Figures

DNA methylation-based classification of ependymomas in adulthood: implications for diagnosis and treatment

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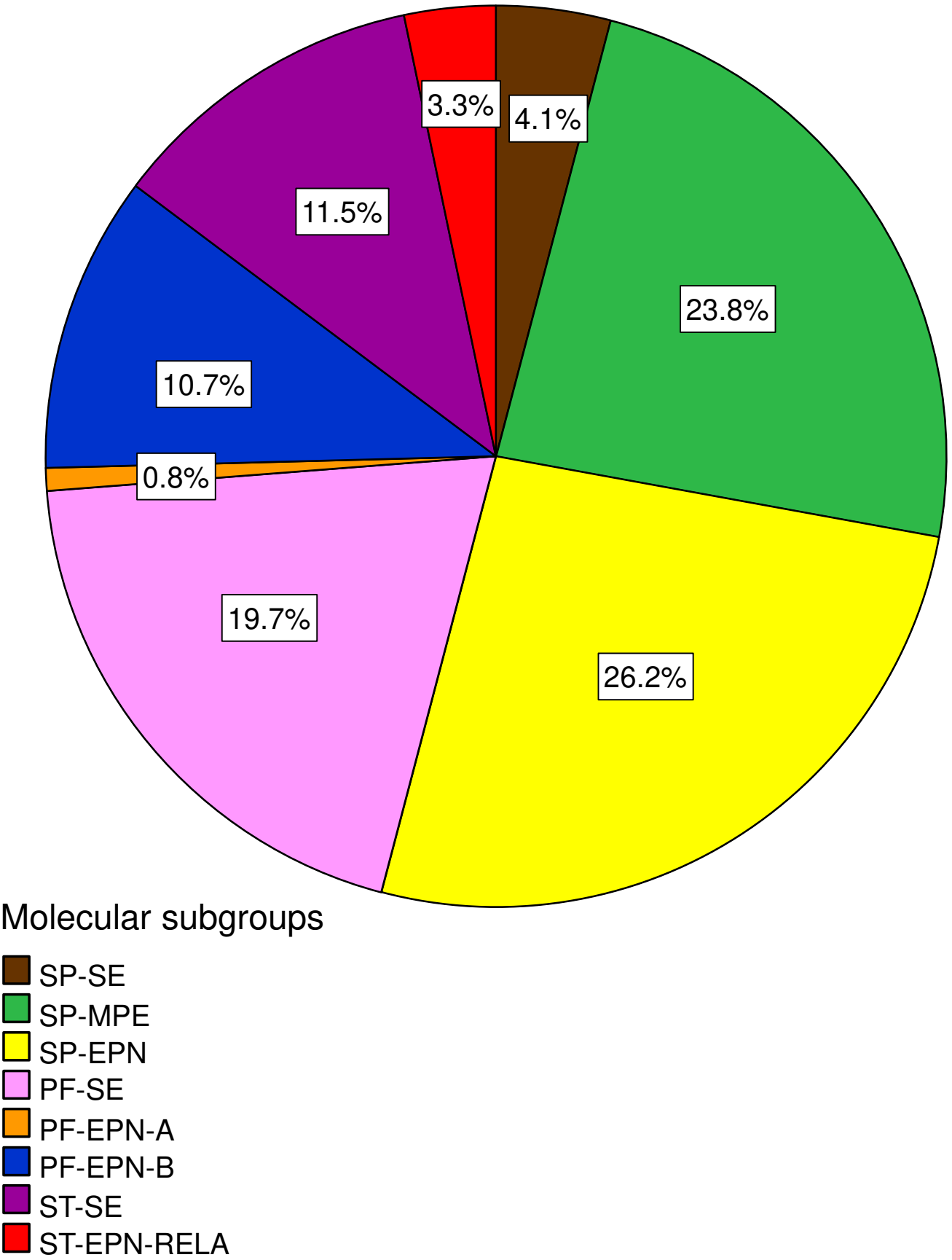
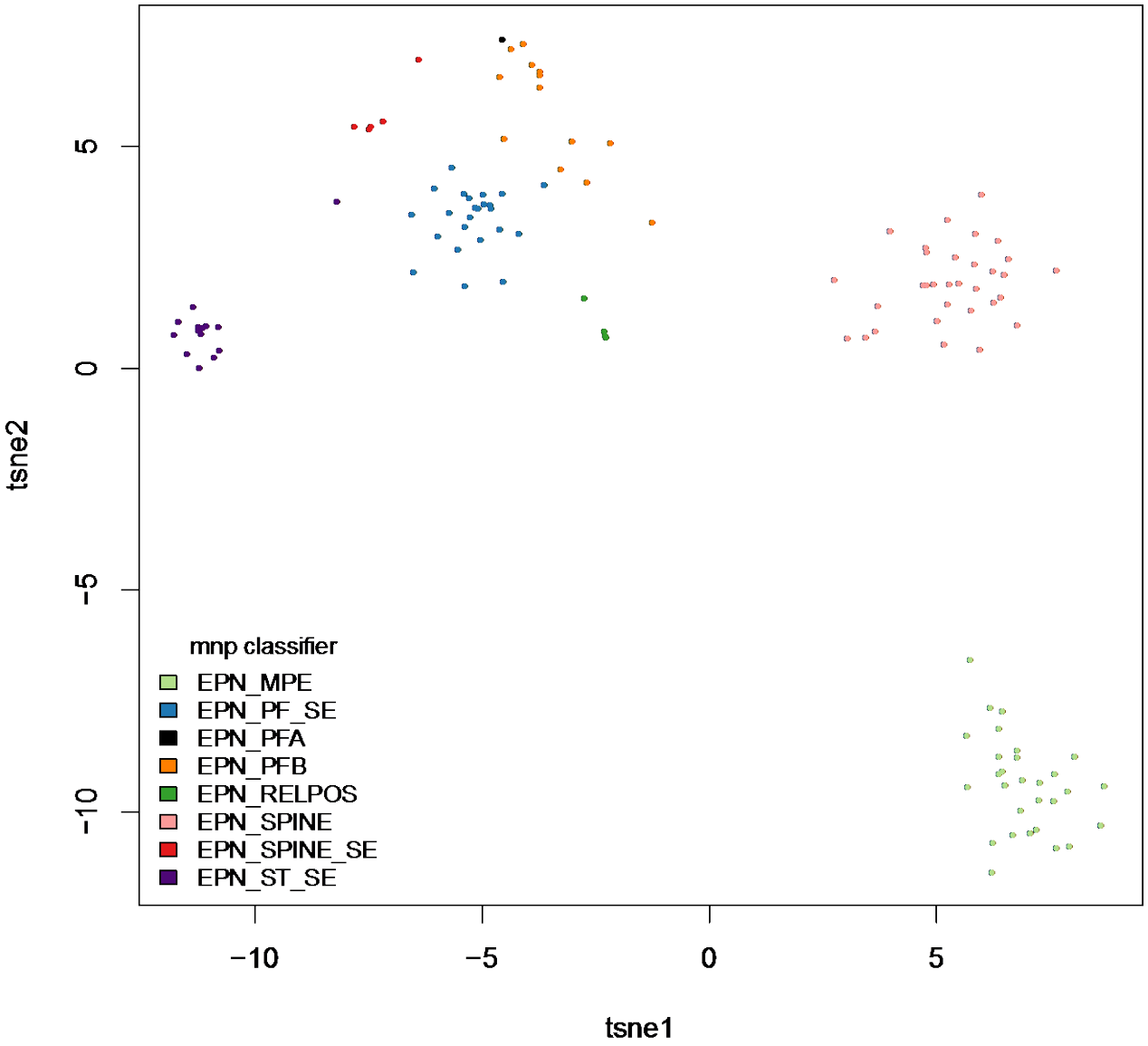
Supplementary Figure 1:



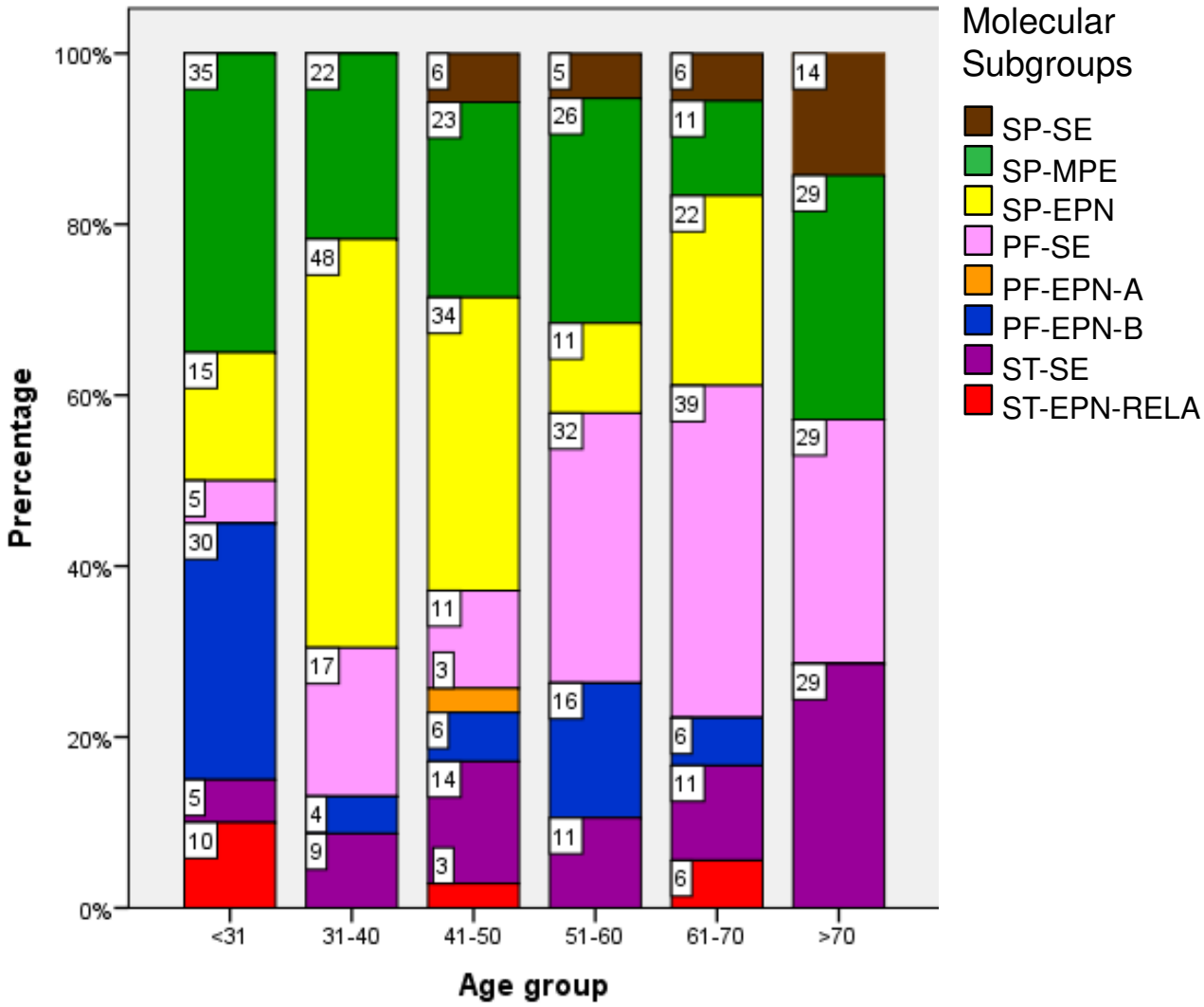
Supplementary Figure 1: Distribution of molecular subgroups of adult ependymal tumors of the German Glioma Network cohort by DNA methylation analysis.

Supplementary Figure 2:



Supplementary Figure 2:
Unsupervised 2D representation of pairwise sample correlations by t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction. Individual samples (n=122) are color coded in the respective class color. Samples of the same class form dense aggregations and are mostly clearly separated from other classes. Other classes (molecular subtypes) like EPN-PF-SE, EPN-PFA, EPN-PFB show a closer aggregation, indicating a higher inter-class relation.

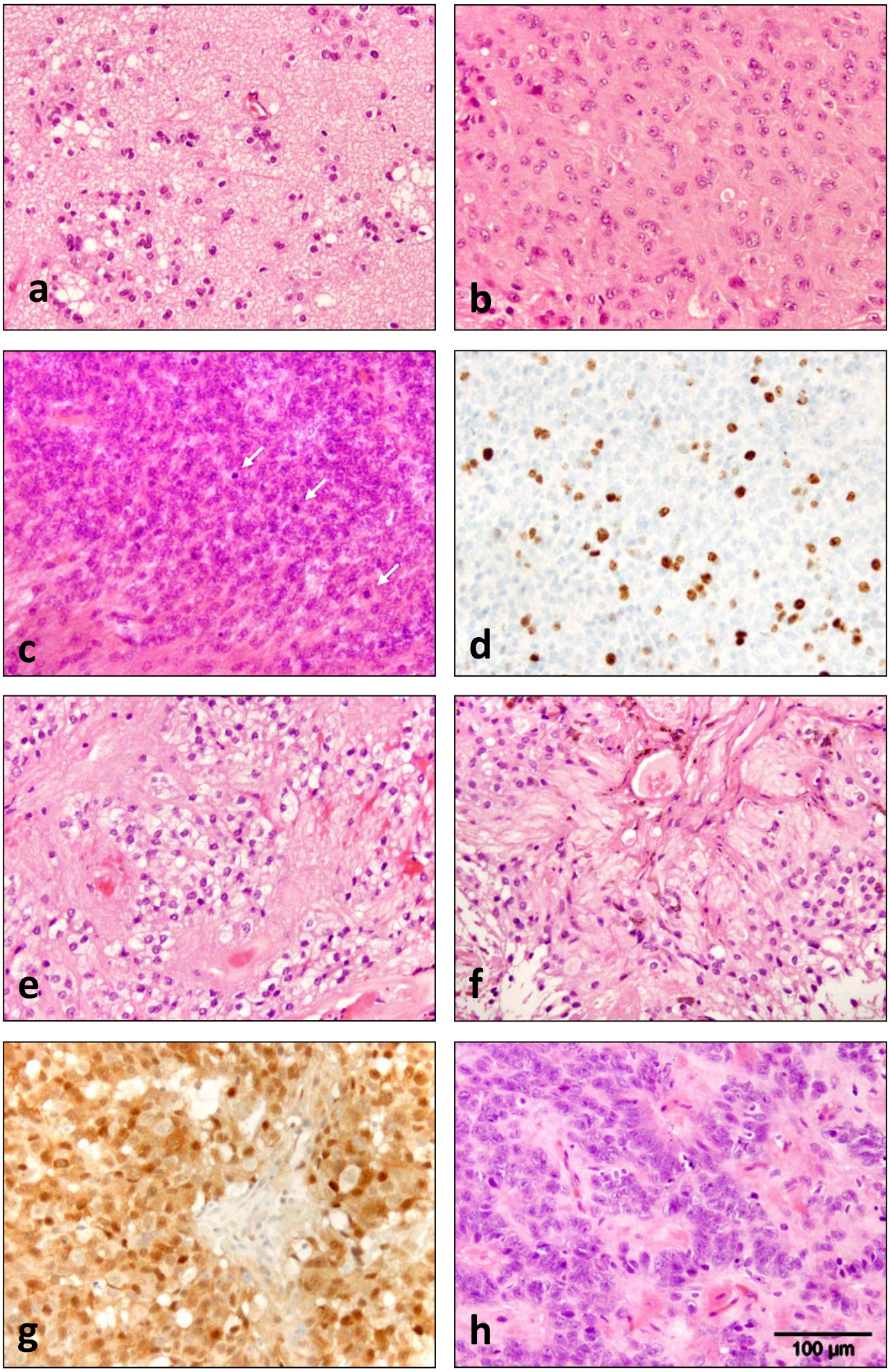
Supplementary Figure 3:



	Age group (years)						
	<31	31-40	41-50	51-60	61-70	>70	Total
SP-SE	0	0	2 (5.7%)	1 (5.3%)	1 (5.6%)	1 (14.3%)	5 (4.1%)
SP-MPE	7 (35.0%)	5 (21.7%)	8 (22.9%)	5 (26.3%)	2 (11.1%)	2 (28.6%)	29 (23.8%)
SP-EPN	3 (15.0%)	11 (47.8%)	12 (34.3%)	2 (10.5%)	4 (22.2%)	0	32 (26.2%)
PF-SE	1 (5.0%)	4 (17.4%)	4 (11.4%)	6 (31.6%)	7 (38.9%)	2 (28.6%)	24 (19.7%)
PF-EPN-A	0	0	1 (2.9%)	0	0	0	1 (0.8%)
PF-EPN-B	6 (30.0%)	1 (4.3%)	2 (5.7%)	3 (15.8%)	1 (5.6%)	0	13 (10.7%)
ST-SE	1 (5.0%)	2 (8.7%)	5 (14.3%)	2 (10.5%)	2 (11.1%)	2 (28.6%)	14 (11.5%)
ST-RELA	2 (10.0%)	0	1 (2.9%)	0	1 (5.6%)	0	4 (3.3%)
TOTAL	20 (100%)	23 (100%)	35 (100%)	19 (100%)	18 (100%)	7 (100%)	122 (100%)

Supplementary Figure 3: Molecular subgroups by age distribution.

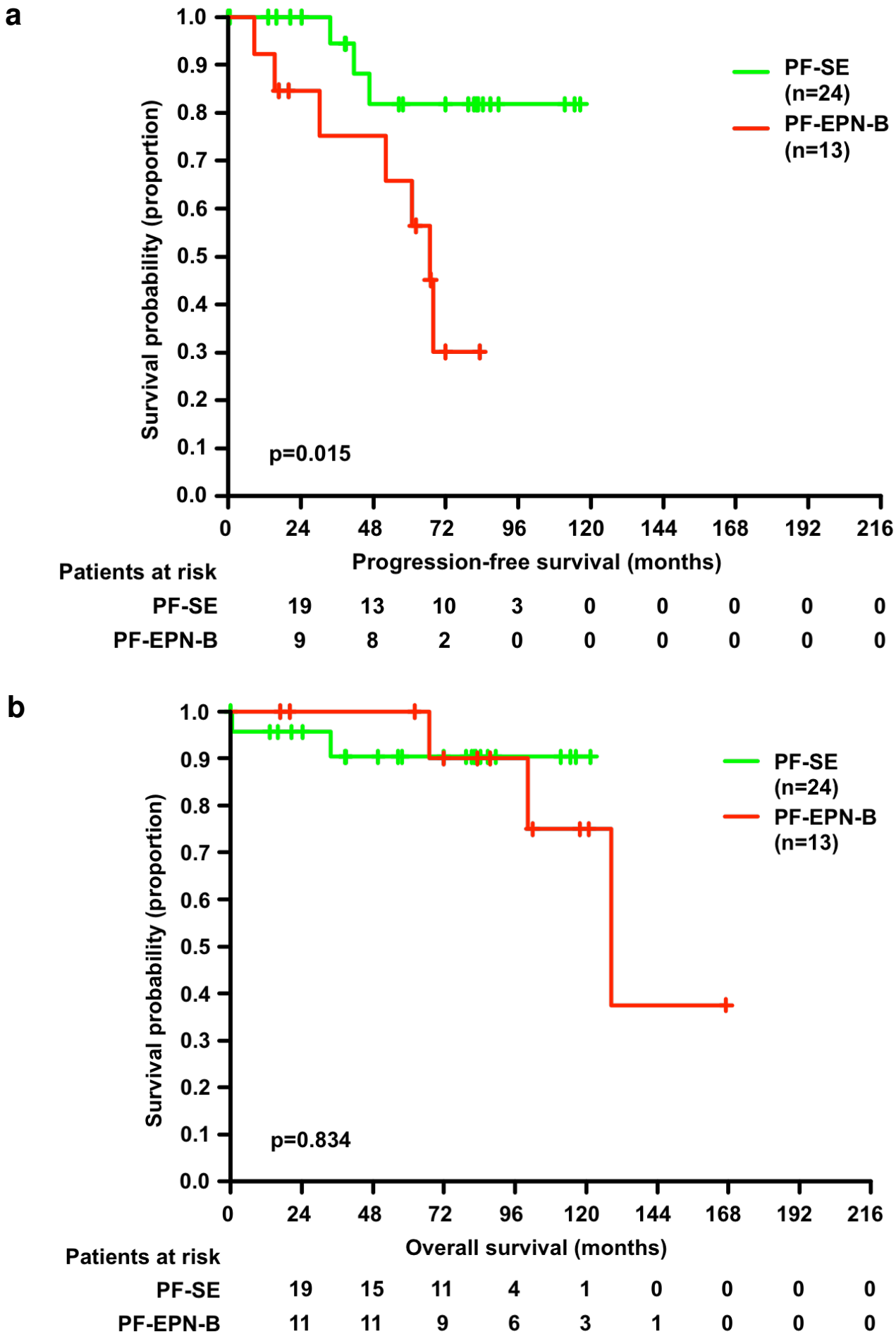
Supplementary Figure 4:



Supplementary Figure 4: **Histological versus molecular group assignment.**

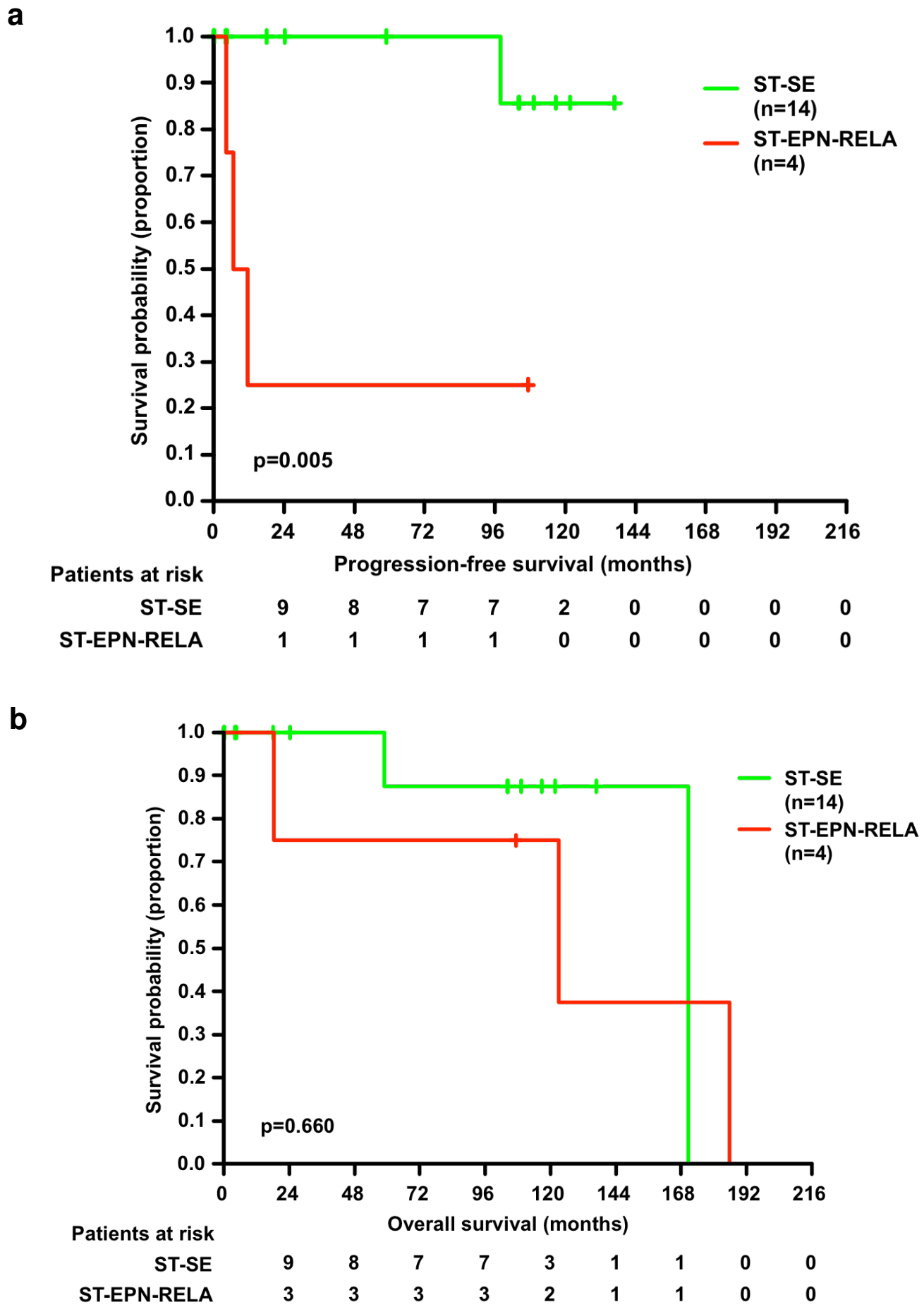
Histology of cases assigned by methylation profiling to the methylation group SE (subependymoma) varies (a-d).
(a) Characteristic features such as clustered low-proliferative cells at low density lead to the WHO diagnosis of subependymoma (WHO grade I). Other cases of this methylation group contained histologically defined ependymoma (WHO grade II) (b) and even a case of high cellularity and brisk mitotic (c, arrows) and proliferative activity (d, KI-67 immunohistochemistry);
Representative histology examples (e – f) show a spinal case that had predominant clear cell features but very focal perivascular mucoid deposits (f). This case was diagnosed as ependymoma WHO grade II although it showed local features of myxopapillary ependymoma, and corresponded to the methylation subgroup of SP-MPE (myxopapillary ependymoma); (g) anaplastic ependymoma with RELA fusion displaying strong nuclear accumulation of p65-RelA protein (immunohistochemistry; *Pietsch et al, 2014* [7]);
(h) shows the only posterior fossa tumor defined by methylation classifier as EPN-PFA tumor. Histologically, it showed advanced differentiation and very low mitotic activity, and was diagnosed as ependymoma WHO grade II.

Supplementary Figure 5: Posterior fossa Subgroups



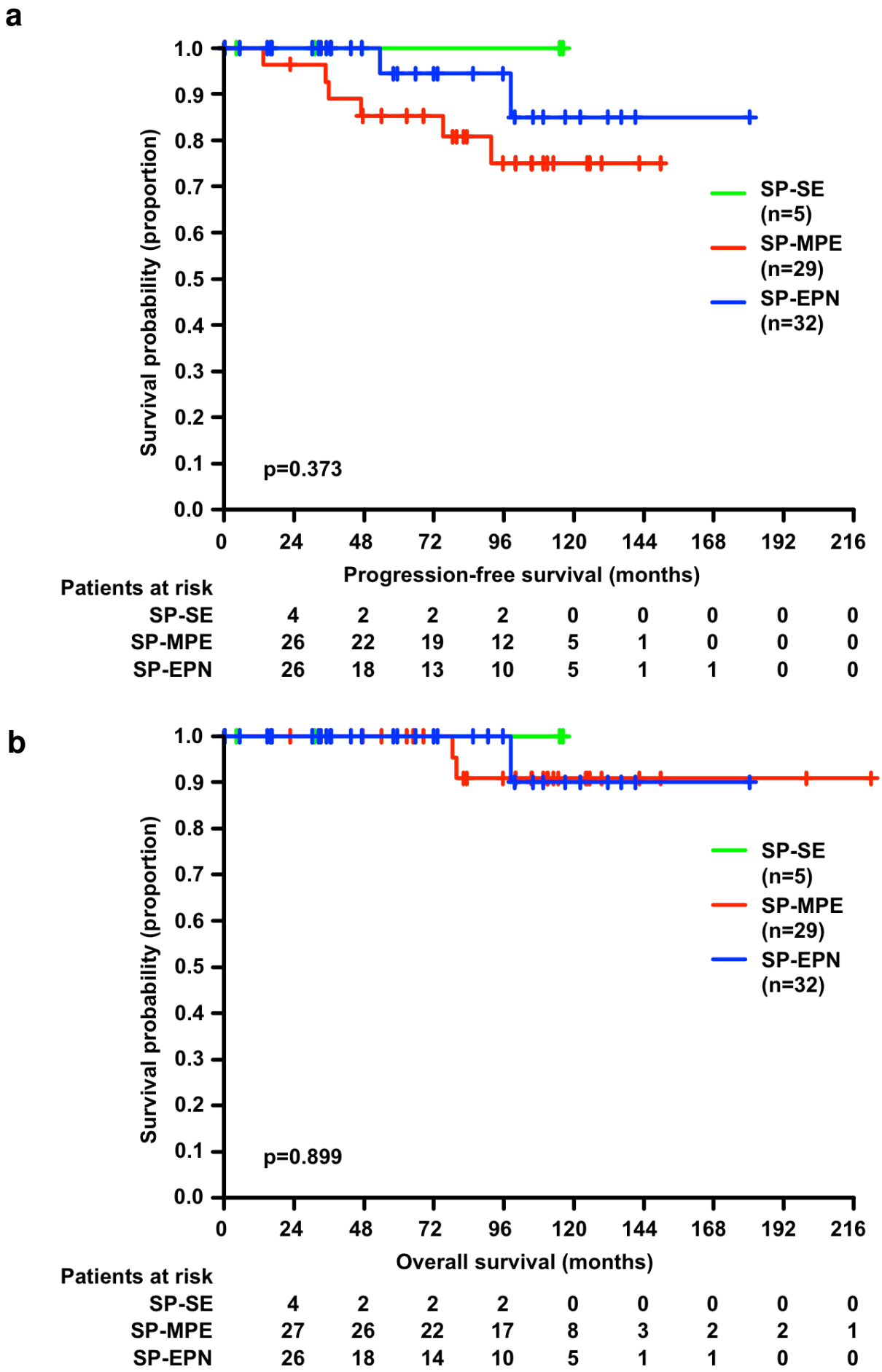
Supplementary Figure 5: **Inferior outcome with PF-EPN-B tumors.**
PFS (a) and OS (b) for patients with PF-SE and PF-EPN-B tumors.

Supplementary Figure 6: Supratentorial Subgroups



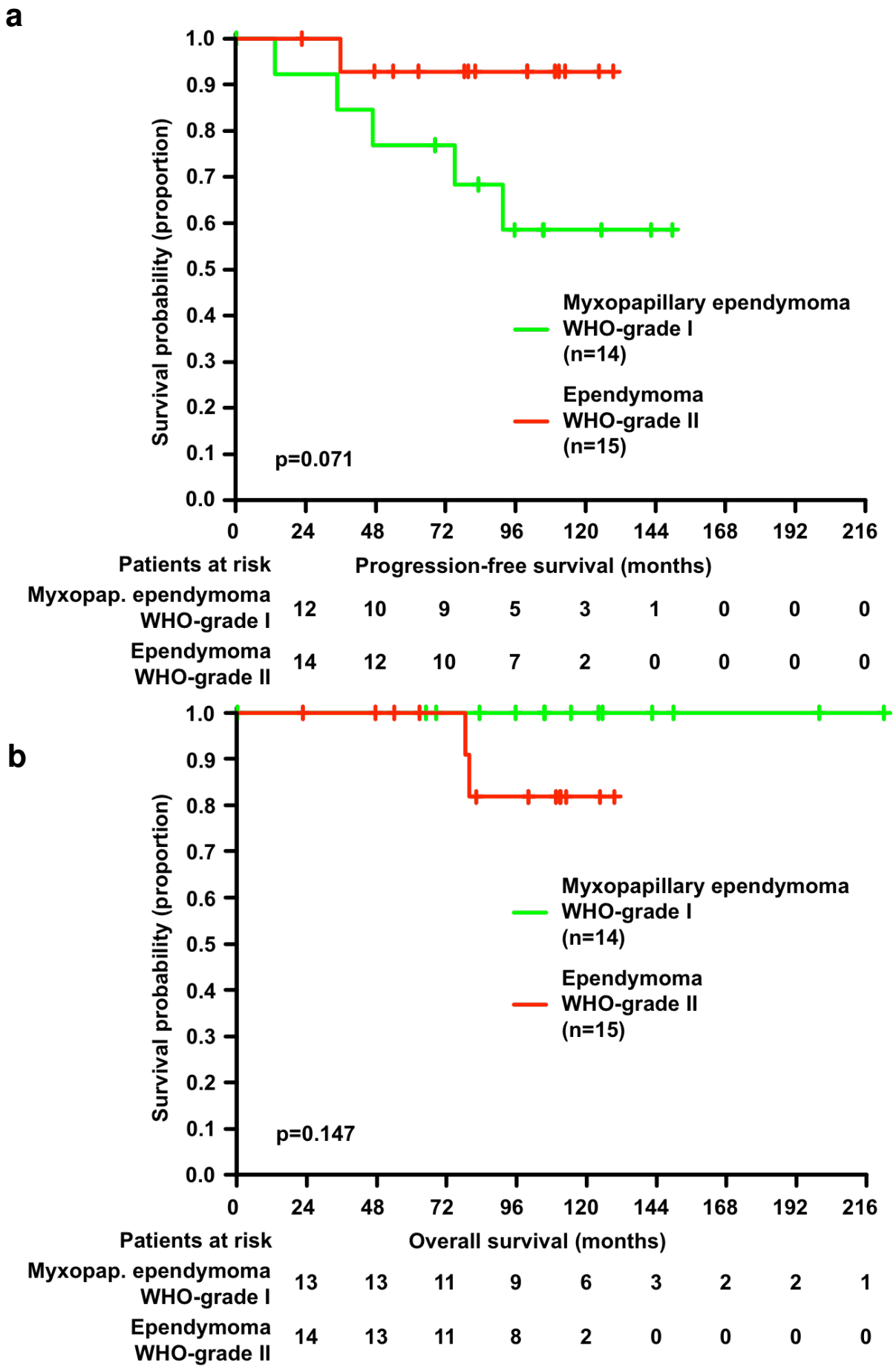
Supplementary Figure 6: **Inferior outcome with ST-EPN-RELA tumors.**
PFS (a) and OS (b) for patients with ST-SE and ST-EPN-RELA tumors.

Supplementary Figure 7: Spinal Subgroups



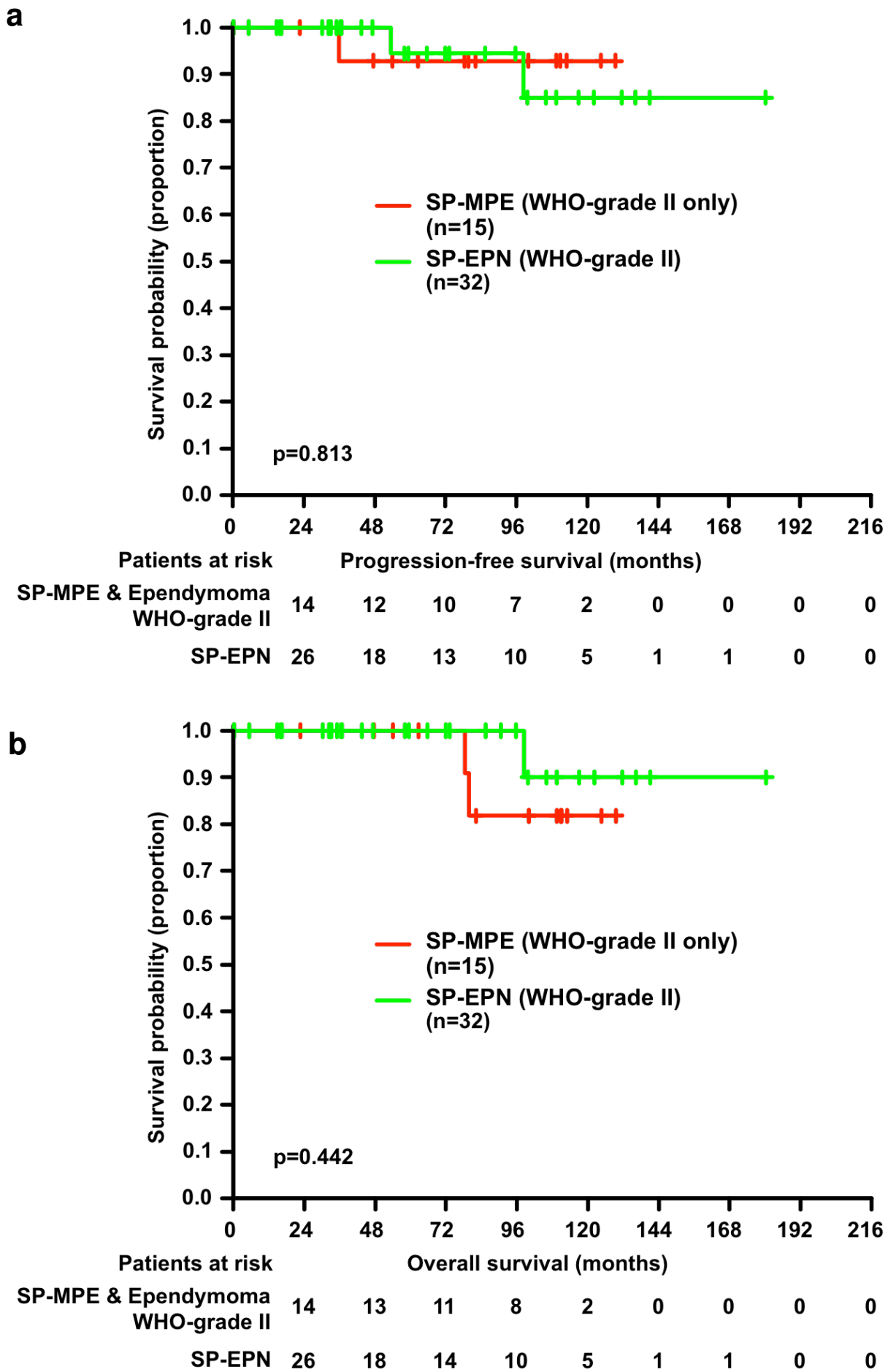
Supplementary Figure 7: **Outcome of spinal ependymomas by molecular subgroup.** PFS (a) and OS (b) for patients with SP-SE, SP-MPE and SP-EPN tumors.

Supplementary Figure **8:**



Supplementary Figure **8:** **Outcome of SP-MPE tumors by histology.** PFS (a) and OS (b) for patients with histological myxopapillary ependymoma WHO grade I versus ependymoma WHO grade II

Supplementary Figure 9:



Supplementary Figure 9. **Outcome of histological spinal WHO grade II ependymoma by molecular assignment.** PFS (a) and OS (b) for SP-MPE versus SP-EPN tumors.